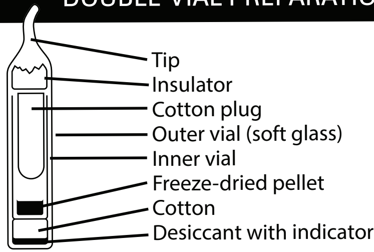
Reviving Freeze-Dried Microorganisms
Instructional Guide

ATCC®

INTRODUCTION

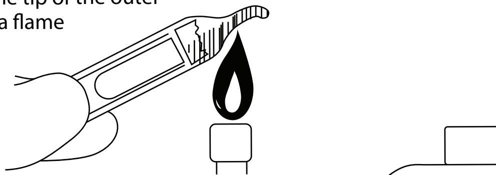
- All cultures should be regarded as potentially hazardous and should be opened by persons trained in microbiological techniques working in facilities with containment requirements appropriate for the biosafety level of the cultures.
- Work in a biological safety cabinet. If this is not possible, wear suitable eye protection. Hold vials away from your face and over a can or tray.
- Wear gloves.
- After sterilizing vials with alcohol, commercially available glass tubing cutters may also be used to open vials.
- Sterilize all empty vials and fragments before disposal.

DOUBLE-VIAL PREPARATIONS

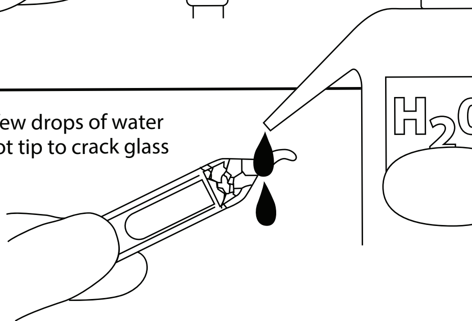


OPENING THE VIAL

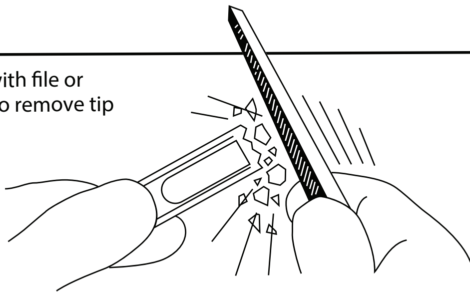
1 Heat the tip of the outer vial in a flame



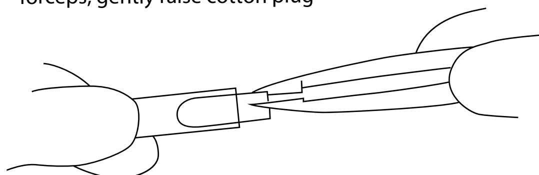
2 Squirt a few drops of water on the hot tip to crack glass



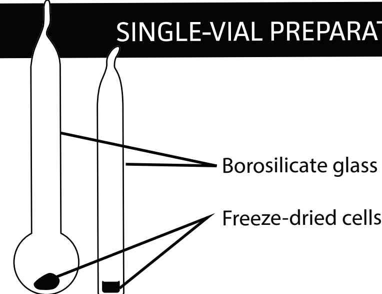
3 Strike with file or pencil to remove tip



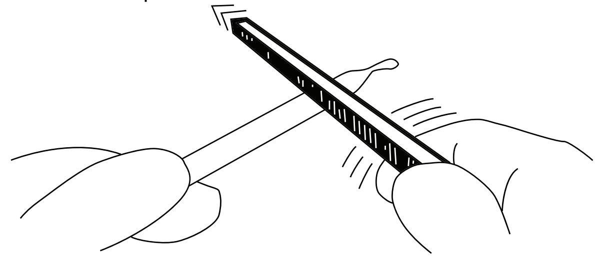
4 Remove insulation and inner vial with forceps, gently raise cotton plug



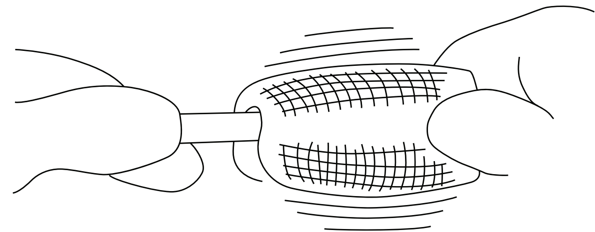
SINGLE-VIAL PREPARATIONS



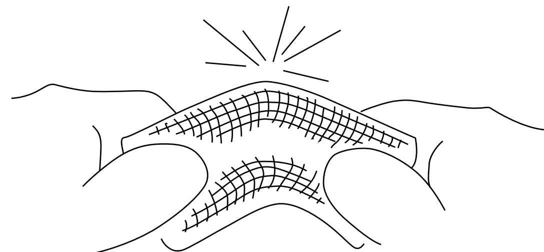
1 These preparations may be enclosed in a thin skin of cellulose; this skin must be removed (either with a sharp blade or by soaking in water for a few minutes). Score the ampule once briskly with a sharp file about one inch from the tip.



2 Disinfect the ampule with alcohol-dampened gauze



3 Wrap gauze around the ampule, and break at the scored area. Care should be taken not to have the gauze too wet, or alcohol could be sucked into the culture when the vacuum is broken. Rehydrate material at once.



Check each culture thoroughly upon receipt. If you received a double glass vial, inspect the blue crystal desiccant (silica beads) in the bottom of the outer vial. If the desiccant is clear or pink, the vacuum seal may have been compromised and the material may not be viable. If a culture is unsatisfactory, notify ATCC so that the strain in question can be investigated. Store freeze-dried cultures at 2°C to 8°C or colder if they are not immediately rehydrated (except plant viruses, which should be stored at -20°C). Use the medium and incubation conditions specified on the product sheet when first reviving strains to ensure optimal conditions for recovery. The product sheet, certificate of analysis, and media formulations for each product are available online on the respective product detail page at www.atcc.org. Additional technical information on the growth and handling of microorganisms can be found in the ATCC Culture Guides, which are available online at www.atcc.org/guides.

BACTERIA AND ALGAE

The preferred method for long-term preservation of bacteria and algae is freeze-drying; however, some bacteria do not survive freeze-drying well and are frozen instead. For freeze dried cultures, using a single tube of the recommended media (5 to 6 mL), withdraw approximately 0.5 to 1.0 mL with a Pasteur or 1.0 mL pipette. Use this to rehydrate the entire pellet, and transfer the entire suspension back into the broth tube and mix well. The last few drops of this suspension may also be transferred to an agar slant. Alternatively, algal cultures must be initiated on agar plates. Please note that anaerobic bacterial cultures must be rehydrated in an anaerobic environment; the viability of the cells decrease rapidly if the vial is rehydrated in an oxygenic environment.

Incubate cultures under the appropriate conditions. Given proper treatment and conditions, most freeze-dried cultures will grow out in a few days. However, some may exhibit a prolonged lag period and should be given twice the normal incubation time before discarding as nonviable.

BACTERIOPHAGES

Prior to rehydrating the phage, prepare an actively growing broth culture of the bacterial host. Rehydrate the freeze-dried phage specimen aseptically with 1.0 mL of appropriate broth (refer to the product sheet) and mix well. Use 0.1 mL of this mixture for the preparation of a new high-titer phage suspension. Preserve the remaining mixture in a sterile screw-capped vial at 2°C to 10°C. Refer to the product sheet for specific information on how to propagate the phage.

FILAMENTOUS FUNGI AND YEAST

Prior to rehydrating your fungi, refer to the product sheet for any specific instructions regarding the handling of your culture. For freeze-dried fungi, use a Pasteur pipette to add approximately 0.5 to 1.0 mL sterile water to the inner vial of a double vial or to a serum vial (Preceptrol®). Then, draw up the entire contents into the pipette and transfer to a test tube with about 5 to 6 mL sterile water. Let the yeast or fungus rehydrate for at least a couple of hours before transferring to broth or solid agar; longer rehydration (*e.g.*, overnight) might increase the viability of some fungi. Incubate at the recommended temperature. Keep in mind that some cultures may exhibit a prolonged lag period and should be given twice the normal incubation time before discarding as nonviable. Save the mixture of lyophilized material and water until you know you have growth.

PLANT VIRUSES

Plant viruses are usually distributed in freeze-dried plant tissues within single, sealed vials. For increased stability of the contents, vials should be stored at -20°C prior to use. Vials should be opened carefully, removing the metal retaining cap and the rubber stopper. However, if the vial has been flame-sealed, it may be opened according to the directions provided on back.

For tissue reconstitution and inoculum preparation, the contents of the vial should be placed in a precooled (4°C) mortar with 2 to 3 mL of an inoculation buffer (*e.g.*, 0.05 M sodium phosphate buffer, pH 7.0, with 10 mM sodium sulfite). A pestle is used to thoroughly triturate the tissue for inoculum preparation. The inoculum may be rubbed onto host plants using a sterile cotton swab and a fine abrasive, such as 500 to 600 mesh carborundum (silicon carbide) or celite (diatomaceous earth). The abrasive may be added to the inoculum (50 to 100 mg/mL) or dusted onto the plants prior to inoculation. After inoculation, the plants should be sprayed with water to remove buffer salts and abrasive.

CULTURES IN STOPPERED SERUM VIALS

Freeze-dried bacteria and fungi supplied in stoppered serum vials (Preceptrol® cultures) should be opened carefully by aseptically removing the metal retaining cap and the rubber stopper. To propagate, follow instructions for the appropriate type of organism.

If you have questions, please contact a technical service representative at **tech@atcc.org** or 800-638-6597 (703-365-2700), or contact your local distributor. Details regarding our warranty can be found in the Material Transfer Agreement packed in your shipment or available at **www.atcc.org.**

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